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ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF A NIGERIAN POLYHERBAL TONIC TEA (PHT) EXTRACT IN RODENTS

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Abstract

The study investigated the anti-nociceptive and anti-inflammatory properties of a Nigerian Polyherbal Health Tonic tea aqueous extract (PHT) in rodents of both sexes. 100 - 500 mg kg^{-1} of the aqueous extract was administered via the intra-peritoneal (i.p.) and oral (p.o.) routes to 5 groups of mice using tail immersion, tail clip, formalin and acetic acid –induced writhing tests of experimental nociceptive models. Each of the models showed that PHT possesses a significant ($p<0.05$) anti-nociceptive effects which were peripherally and centrally mediated as both the early and late phases of pain significantly ($p<0.05$) were inhibited. However, the peripherally mediated analgesic effect of PHT, although similar to that of aspirin but was found to be more potent than aspirin. In assessing its anti-inflammatory potentials, 300 - 1340 mg kg^{-1} PHT was also administered via oral and intraperitoneal routes, which, significantly ($p<0.05$) reduced the volume of carrageenan-induced oedema. Although, PHT administered via i.p. route was more effective than the oral but there was barely any difference between the percentage inhibition of oedema volume at both 600 and 1340 mg kg^{-1} given orally. PHT anti-inflammatory effect was elucidated to be significantly ($p<0.05$) mediated via histaminergic, serotonergic, bradykinin and prostaglandin inhibition. PHT was also shown to be more protective than acetylsalicylic acid (ASA) in the castor oil- induced diarrhea model, which suggests the involvement of other mechanisms. Thus, lending supports to its folkloric use in pain and swelling management.

Key words: Polyherbal Health Tonic tea (PHT), Anti-nociception, Anti-inflammation; Oral and intraperi- toneal routes, Rodents

Introduction

Inflammation is a complex series of events that occurs when any tissue or organ is injured or damaged by chemicals, micro-organisms, trauma, foreign bodies, surgery and ionizing radiation (Foster, 1999). It involves the releases of different vasoactive substances such as histamine, serotonin and the prostaglandins (Jackson-Robert II and Morrow, 2001). Conversely, pain, is the sensation of discomfort, hurt or distress often accompanying inflammatory responses and usually result from local stimulation of pain fibers and hyperalgesia, and in part a consequence of increased excitability of central neurons in the spinal cord (Jackson-Robert II and Morrow, 2001).

Medicinal plants play a key role in the human and animal health care. About 80% of the world population is being documented to rely on the use of herbal medicine (WHO, 1993). The classical analgesic drugs, opiates and non-steroidal anti-inflammatory drugs (NSAIDs) all have their origin in natural products that have been used for centuries. Salicin, a bitter glycoside from the willow bark extract has been known since the 18th century for its beneficial effect in fever and pain. The synthetic form, acetylsalicylic acid (ASA) was introduced into medicine as far back as the 19th century. Quite a number of derivatives of ASA and other newer drugs have since been

discovered but these are associated with untoward effects limiting their use. These factors include allergy, gastric mucosal irritation and/or gastric ulceration due to the acidic nature of most NSAIDs and inhibition of a mucosal protective prostaglandin E (PGE). Others include prolonged vascular bleeding, NSAID-induced nephropathy, salt and water retention by the kidney as well as displacement of other drugs from their protein binding sites due to the greater affinity of NSAIDs for plasma albumin are some of the other challenges facing this group of drugs (Foster, 1999). Thus, the search for an ideal NSAID continues.

Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. The global search involves investigating single plant extracts or fractions thereof or mixtures of fractions extracts from different plants, which have been carefully standardized for their safety and efficacy (Subbramoniam and Pushpangadan, 1999). In Nigeria, dependence on herbal drug use is also increasing (Nwabuisi, 2002). The polyherbal tea studied in the present study is one of several indigenous polyherbal formulae used in the treatment of diverse human diseases in Nigeria. The formula is a composition of the dried pulverized leaves of *Persea africana* (Moraceae), *Mangifera indica* (Anacardiaceae), *Morinda lucida* Benth. (Rubiaceae), *Carica papaya* (Annonaceae), *Vernonia amygdalina* (Compositae), and *Cassia occidentalis* L. (Caesalpiniaceae), all combined in equal weight ratio of 9 g per packet. The therapeutic claims made by the users of PHT in the treatment of various human illnesses including pain aroused our interest to scientifically validate its analgesic, anti-inflammation, and anti-diarrhoeal properties as well as its mechanisms of action in rat models *in vivo*.

Materials and Methods

PHT purchase and preparation of its aqueous extract

Three 60 g packets of PHT were purchased from a local herbal drug shop in Lagos metropolis. The boiled aqueous extract was used throughout in the study. From a 30 g of PHT which was boiled in 500 mL of distilled water for 1 hour, 3.37 g of chocolate coloured solid residue was obtained, giving a percentage yield of 11.2%. The residue, thus, obtained was stored in a capped container, kept at 4^{o}C until required for use. From this, various concentrations were reconstituted in a known volume of distilled water before administration.

Experimental Animals

Experiments were carried out on Sprague - Dawley rats weighing 100-170 g and Swiss albino mice, 18 - 20 g, which were bred in the College Laboratory Animal Centre. The animals were fed *ad libitum* with rodents chow and had free access to drinking water, but fasted 12 h prior to experimentation. They were kept in a room with controlled 12 h light/dark cycle (6.00 – 18.00) and temperature (25 - 27^{o}C). All experiments were performed between 9.00 and 18.00 h. All experimental procedures were performed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as contained in United States National Institutes for Health Guidelines (1985).

Drugs

Morphine, acetylsalicylic acid, (ASA) carrageenan, formalin and acetic acid, histamine, serotonin (Sigma Chemical Co., St. Louis, U.S.A.; bradykinin (Sandoz AG, Basel/Schwetz, Switzerland), castor oil (Bells Products, Madrid, Spain were used in the experiment. Other drugs were of analytical grade purchased locally. Oral doses and intraperitoneal injections were administered in volumes 10 mL kg $^{-1}$ and 10 - 20 mL kg $^{-1}$ of body weight of animals, respectively

Phytochemical test

Phytochemical analyses of PHT for its active biological principles were conducted using the standard methods described by Farnsworth (1989) and Sofowora (1993).

Anti-nociceptive activity

a. Tail Clip method

All the mice were screened by applying a metal artery clip to the base of the tail. The pressure exerted by the clip was so adjusted that it was just sufficient to make all control mice respond. The animals that did not attempt

to dislodge the clip within ten seconds were not used for the experiment. The selected mice were divided into five groups of six animals each. The tail clip was applied 0, 30, 60, 90, 120 and 150 min. after PHT administration at 150 - 350 mg kg^{-1}/i.p. and 500 mg kg^{-1}/ oral dose routes. The doses of PHT used were determined from the preliminary study done. Morphine (10 mg kg^{-1}, subcutaneous) and distilled water (10 mL kg^{-1}, i.p) were also separately given to positive and negative control groups. A positive analgesic response was indicated if there was no attempt to dislodge the clip within 10 seconds in any of the four consecutive trials after a time period of two minutes and the mean value was taken.

b. Tail Immersion Method

The central analgesic activity of PHT was evaluated using method of Turner (1965). Mice were divided into five groups of six animals each after initial screening. 10 mL/kg of distilled water (control), 150 - 500 mg kg^{-1} PHT and morphine were administered as described for the tail clip test. The tail (up to 5 cm) was then dipped in a water bath maintained at 55 ± 0.5 ^{o}C. The time (in seconds) it takes to withdraw the tail clearly out of the water was taken as the reaction time. The first reading (0 min) was taken immediately after administration of the test drugs and subsequently taken at time 30, 60, 90, 120 and 150 min.

c. Acetic Acid-induced Writhing Test

The test extract and reference drug (acetylsalicylic acid, 100 mg kg^{-1}) were administered orally to groups of mice. After 1 h, 15 mL kg^{-1} of 0.6% acetic acid was injected i.p. Nociception or algesia was evaluated by counting the number of abdominal constrictions in 15 min after acetic acid injection.

d. Formalin Test

Acetic acid-induced writhing is a highly sensitive but not a very selective pain test since false positives occur with sedatives, muscle relaxants and other pharmacological activities. The formalin test in mice is sensitive to non-steroidal anti-inflammatory drugs and other mild analgesics. The test possesses two distinctive phases, possibly reflecting different types of pain. The earlier phase reflects direct effect of formalin (non-inflammatory pain) on nociceptors, whereas, the late phase reflects inflammation (Hunskaar and Hole, 1987). 30 µL of 1% formalin were injected into the dorsal surface of the right hind-paw. The time spent in licking and/or biting responses of the injected paw was taken as an indicator of pain response.

Anti-inflammatory Activity
a. Carrageenan-induced paw oedema

Inhibition of the carrageenan-induced hind paw oedema was used as a measure of anti-inflammatory activity. Groups of animals (n = 6) received 300 mg – 1340 mg $kg^{-1}$of the herbal tea, both orally and intraperitoneally. The other two groups which served as positive and negative controls were administered 100 mg kg^{-1}ASA and distilled water, respectively. One hour after drug administration, oedema of the rat hind paw was produced by injecting into the sub-plantar surface, 0.5% w/v carrageenan suspension. Increase in the linear paw diameter was taken as an index of index of the paw volume which was measured on a linear scale immediately before injecting the carrageenan and at hourly intervals thereafter up till 5 h. The volume of oedema was expressed for each animal as the difference in the diameter of the rat paw before and after injection of the carrageenan. The results are documented as percentage anti-inflammatory activity for the difference in oedema diameter obtained using a standard formula (Bamgbose and Naomesi, 1981; Mascolo et al., 1987).

b. Inflammatory mediators-induced paw oedema

In another set of experiment, different inflammatory mediators (phlogistic) agents were use as oedemogens (Parma and Ghosh, 1978). The respective strength of the oedemogens, the volume injected and the time of oedema volumes are indicated in parentheses: histamine (10^{-3} g mL^{-1}, 0.1 mL, 60 min), serotonin (10^{-3} g mL^{-1}, 0.1 mL, 30 min), and bradykinin (2×10^{-5} g mL^{-1}, 0.1 mL, 60 min) were all injected into the right hand paw of the rat 30 min post- PHT treatment and control vehicle (10mL kg^{-1} distilled water, i.p.) injected into the groups of rats. The oedema volume was determined as earlier stated.

Castor oil induced-diarrhea

This procedure investigates the mechanism that might account for the anti-inflammatory and related action of PHT. Doses 300 - 1340 mg kg^{-1} of the extract, 100 mg kg^{-1} aspirin, and control vehicle (10 mL kg^{-1} distilled water, i.p.) was administered 1 h after the administration of castor oil. Rats were examined for presence or absence of characteristic diarrhoeal droppings on a white paper on the floor of their cages every hour for 4 h absence of diarrhoeal dropping was recorded as a positive result indicating possible inhibition of the biosynthesis of prostaglandins.

Statistical analysis

Values reported are mean ± S.E.M. Statistical analysis was done using two-ways analysis of variance on computer-based statistical program, PRIMER 87. This was followed by post-hoc test which was conducted using Student's t-test. Statistical significance was considered at $p<0.05$.

Results

Different phytochemicals were observed to be present in the aqueous extract of the polyherbal tea. Alkaloids were heavily present followed by tannins, phlobatinins, flavonoids, cardiac glygosides and saponins (Table 1).

The antinociceptive effects of PHT compared with standard NSAIDs are summarized in Table 2. At the doses investigated, PHT elicited significant ($p<0.05$) analgesic action with the tail immersion technique, whereas, only 350 and 500 mg kg^{-1}, i.p., were significantly ($p<0.05$) effective in the tail clip method. The tail immersion method produced a moderate but dose-dependent analgesic action with the i.p route. A much reduced effect was recorded with the oral route. Aside investigating its central analgesic effect, the peripheral effect was also studied through acetic acid-induced writhing response in mice. It was found that PHT significantly ($p<0.05$) inhibited the acetic acid induced writhing responses in a dose-dependent manner (Figure 1). Also, PHT effectively reduced the wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limb in mice due to the nociceptive property of acetic acid. In the formalin test, the extract was effective in both early and late phases of formalin-induced pain in a dose dependent fashion. However, as usual, the effect of oral dose was much lower than i.p. (Table 3).

The polyherbal tea significantly ($p<0.05$) reduced the volume of oedema in the carrageenan-induced inflammation. Dose variation of PHT had no significant ($p>0.05$) anti-inflammatory changes in the PHT-treated rats (Table 2). However, the standard drugs, aspirin and indomethacin, produced greater anti-inflammatory effects than PHT. Furthermore, 300 mg kg^{-1} i.p. of PHT studied on selected chemical inflammatory mediators reduced the paw oedema formation induced by the different phlogistic agents- histamine, serotonin and bradykinin (Table 5). The percentage inhibition of edema was highest with bradykinin. In the assessment of the effect of extract on prostaglandins using castor oil-induced diarrhoea method, castor oil produced characteristic semisolid diarrhoeal dropping in all the animals of the control group all through the period of investigation. At the 1h interval, PHT at a dose of 150 mg kg^{-1} i.p. did not prevent diarrhea. A similar trend was observed with 500 mg kg^{-1} oral dose. Only 33.3% animals were protected with 300 mg kg^{-1} dose whereas, 100 mg kg^{-1} aspirin offered total protection. At 2 h interval, the i.p. doses offered 100% protection which was sustained throughout the period of investigation. On the other hand, only 66.6% animals were protected by the oral dose between 3 - 4 h of study. ASA could not prevent diarrhoea between 2 - 4 h since 33.3% of the animals produced semi-solid diarrhoeal dropping 2 - 3 h after treatment, while 50% were found to behave similarly at the 4 h interval.

Discussion

The present study aimed at investigating the anti-nociceptive and anti-inflammatory properties of PHT, which has been widely ingested by indigenes for various therapeutic purposes. Series of standard experimental

procedures using various animal models were adapted for this study, since the emotional response to experimental pain in an animal is akin to the human response to disease or accidental injury (Laurence et al., 1997).

In the anti-nociceptive study, the tail immersion, tail clip and the acetic acid-induced writhing tests were used while the formalin-induced edema method was to establish its mechanism(s) of action. The tail immersion technique, which replaced the hot plate model (Uma-Devi et al., 1999), showed a significant ($p<0.05$) dose-dependent effect of PHT although PHT was less effective than morphine (Table 2). Similarly, the tail clip method reported significant anti-nociception with 350 and 500 mg kg^{-1} of extract. The i.p. route at 500 mg kg^{-1} compared effectively with the standard drug at 90 min post-drug administration (Table 2). The above two procedures consists

Table 1: Results of the phytochemical analysis of PHT

Agent/Test	Concentration
Alkaloids	
Dragendorff's test	+++
Mayer's test	+++
Wagner's test	+++
Tannins	
Ferric chloride test	++
Phlobatinnins	
Dilute hydrochloride acid test	+
Flavonoids	
Lead acetate test	-
Sodium hydroxide test	++
Cardiac glycosides	
Kedde's test	++
Salkowski's test	++
Keller-Killiani's test	++
Legal's test	++
Saponin	
Frothing test	++

+ = present in low concentration, ++ = present in moderate concentration, +++ = present in high concentration, - = absent

of behavioural methods that have been developed to study nociception in animals (Ramabadran and Bansinath, 1986). The animal response in these tests is usually integrated at the lower levels in the central nervous system, thus, giving information about the pain threshold. They are, therefore, used to detect narcotic and non-narcotic analgesics, as well as distinguish analgesic from anti-inflammatory properties. It is well established that thermal nociceptive tests are more sensitive to opioid μ-agonists and non-thermal tests to opioid κ-agonists (Abbott, 1988; Furst et al., 1988). Our data suggest the involvement of both κ and μ opioid receptor in the analgesic activity of PHT, from which the central involvement of the extract could be deduced. The formalin - induced pain as an experimental model of analgesia is a useful for elucidating mechanism of pain and analgesia since it measures the response to a long–lasting nociceptive stimulus and, therefore, resembles clinical pain (Murray et al., 1988). Subcutaneous injection of dilute formalin into mice hind-paw produces biphasic nociceptive response namely: Phase 1 reflects an acute pain response due to chemical stimulation of nociceptors while Phase 2 represents the injury-induced spinal sensitization, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron occurs during inflammatory pain (Ashok et al., 2006). Drugs that act centrally, such as the narcotics inhibit both phases of formalin - induced pain, while peripherally acting drugs such as ASA only inhibit the late

Table 2: Inhibitory effects of PHT on Tail immersion and Tail clip Tests in mice

MODEL	TREATMENT AND ROUTE	DOSE (mg kg^{-1})	RESPONSE TIME AFTER DRUG ADMINISTRATION					
			0	+30	+60	+90	+120	+150
Tail immersion								
	DW	10 ml kg^{-1}	0.6 ± 1.3	1.1 ± 2.7	1.1 ± 3.6	1.1 ± 1.1	1.0 ± 0.3*	1.5 ± 2.4*
	PHT (i.p.)	100	0.6 ± 1.6	2.3 ± 4.4*	3.8 ± 5.2*	3.9 ± 2.1*	3.5 ± 1.5*	1.3 ± 0.3*
	PHT (i.p.)	500	1.0 ± 3.5	2.7 ± 3.5*	4.0 ± 2.6*	4.0 ± 2.2*	3.8 ± 2.8*	2.5 ± 6.0*
	PHT (oral)	500	0.6 ± 2.0	1.6 ± 0.9*	2.5 ± 1.0*	2.5 ± 1.7*	2.1 ± 0.2*	0.7 ± 1.1*
	Morphine (i.p.)	10	0.6 ± 1.1	7.8 ± 2.6*	> 10.0 ± 0.0*	> 10.0 ± 0.0*	>10.0 ± 0.0*	> 10.0 ± 0.0*
Tail Clip								
	DW	10 ml kg^{-1}	0.1 ± 2.3	0.1 ± 0.2	0.1 ± 1.9	0.1 ± 2.8	0.1 ± 1.1	0.1 ± 0.3
	PHT (i.p.)	100	0.1 ± 1.4	0.2 ± 3.4	0.2 ± 1.6	0.2 ± 2.8	0.3 ± 2.1	0.2 ± 2.7
	PHT (i.p.)	350	0.2 ± 3.0	0.2 ± 0.1	1.3 ± 5.3	5.3 ± 4.9*	0.2 ± 0.2	0.0 ± 2.1
	PHT (i.p.)	500	0.2 ± 3.6	0.3 ± 1.1	3.5 ± 2.2*	10.0 ± 1.5*	2.2 ± 2.4	0.1 ± 1.0
	PHT (oral)	500	0.2 ± 0.4	0.2 ± 0.2	0.4 ± 3.2	2.2 ± 2.3	0.5 ± 3.7	0.1 ± 1.2
	Morphine (i.p.)	10	0.1 ± 4.8	10.0 ± 0.0*	10.0 ± 0.0*	10.0 ± 0.0*	10.0 ± 0.0*	7.0 ± 1.9

DW = distilled water; i.p. = intraperitoneal route of administration
*significant value at $p<0.05$ when compared to control
Each value represents the mean ± S.E.M. from six animals in each group

Table 3: Effects of PHT on the early and late phases of formalin-induced pain in rat

Treatment	Dose (mg kg^{-1})	Licking (0-5 min)	Inhibition (%)	Licking (15-30 min)	Inhibition (%)
Control	10 ml/kg NS	103.3 ± 2.4	0.0	99.0 ± 9.0	0.0
PHT (i.p.)	150	67.7 ± 2.3*, **	34.5	12.3 ± 1.5*, **	87.6**
PHT (i.p.)	250	55.1 ± 1.9*, **	46.7	0.0 ± 0.0*, **	100.0**
PHT (oral)	500	76.5 ± 6.9*	25.9	46.5±0.7*	53.0*
ASA (oral)	100	76.3±4.93*	26.1	45.4±1.9*	54.1*

NS = normal saline; ASA = Acetylsalicylic acid; i.p. = intraperitoneal route
* significant values ($p<0.05$) when compared to control value, **significant values ($p<0.05$) when compared to ASA; values are expressed as mean ± S.E.M. of six rats

Table 4: Effects of PHT, aspirin and indomethacin on carrageenan-induced inflammation in rats

Treatment	Dose (mg kg^{-1})	edema diameter after 4h ± S.E.M.	% inhibition
Control (DW) (oral)	10 mL kg^{-1}	3.25 ± 0.02	0.00
PHT (oral)	600	2.42 ± 0.02*, **	75.6*
PHT (oral)	1340	2.37 ± 0.03*, **	77.8*
PHT (i.p.)	300	2.29 ± 0.01*, **	88.9*
PHT (i.p.)	600	2.20 ± 0.01*	91.1*
Aspirin (i.p.)	100	2.27 ± 0.01*	93.6*
Indomethacin (i.p.)	8.3	2.20 ± 0.02*	94.4*

DW = distilled water, i.p. = intraperitoneal route of drug administration
* significant values ($p<0.05$) when compared to control value, **significant values ($p<0.05$) when compared to ASA; values are expressed as mean ± S.E.M. of six rats

Table 5: Effect of PHT on histamine, serotonin and bradykinin-induced paw edema in rats

Group	Dose (mg kg^{-1})	Mean edema volume (mL ± S.E.M.)		
		Histamine	Serotonin	Bradykinin
Control (DW)	10 mL kg^{-1}	0.7 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
PHT	300 (i.p.)	0.3 ± 0.0*	0.20 ± 0.0*	0.1 ± 0.0*
Percent inhibition (%)		54.5*	50.0*	66.7*

DW = distilled water
* significant values ($p<0.05$) when compared to control value
Values are expressed as mean ± S.E.M. of six rats

Table 6: Effect of PHT on castor oil – induced diarrhea in rats

Group	Dose (mg kg^{-1})	% Protection from diarrhea			
		1 h	2 h	3 h	4 h
Control, Castor oil (orally)	10 mL kg^{-1}	0.0	0.0	0.0	0.0
PHT (i.p) + Castor oil (orally)	150	0.0	100.0	100.0	100.0
PHT (i.p) + Castor oil (orally)	300	33.3	100.0	100.0	100.0
PHT (oral) + Castor oil (orally)	500	0.0	33.3	66.6	66.6
ASA +Castor oil (orally)	100	100.0	66.6	66.6	50.0

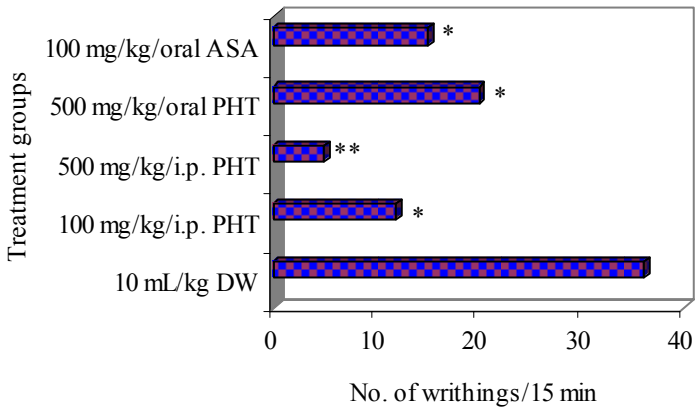

Figure 1: The effects of distilled water (DW), graded doses and routes of administration for PHT and acetylsalicylic acid (ASA) on acetic acid – induced writhing test in mice. *, ** represent significant decreases at $p<0.05$ and $p<0.001$ when compared to values for the control and ASA groups, respectively; n = 6 mice per group

phase (Santos et al., 1994). Our results showed PHT to have inhibited both phases of formalin induced pain (Table 3).Thus, suggesting its central and peripheral anti-nociceptive actions. Aside this, all the drugs produced greater inhibition of second phase than the first but PHT anti-nociceptive effect on phase 2 was more efficacious than the standard drug, ASA. Earlier studies have shown that substance P participates in the early phase, while histamine, serotonin, excitatory amino acids and prostaglandins are involved in the late phase of formalin test with bradykinin affecting both phases (Doak and Sawynok, 1997).

The abdominal contraction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics and such a response is thought to involve local peritoneal receptors. Significant protection was observed in PHT - treated groups of animals and it compared favorably with the standard drug (Fig. 1). ASA is known to produce analgesic effect through inhibition of prostaglandin synthesis, a central component to NSAID – produced analgesia has been described, but the mechanisms underlying it is complex (Jackson-Robert II and Morrow, 2001).

In screening for the anti-inflammatory potential of PHT, 1.0% carrageenan suspension injected into the subplantar paw surface of the groups of rats, induced significant reduction in oedema volume in all the groups treated and favourably compared with aspirin and indomethacin. A striking observation among the orally treated groups was the marginal difference in percentage inhibition between the group administered 600 and 1340 mg kg^{-1}, which suggests that PHT could be largely susceptible to first pass effect (Table 4). Carrageenan-induced inflammation has been known to involve three distinct phases of mediator release, including histamine and serotonin in the first phase, kinins in the second phase and prostaglandins in the third phase (Di Rosa et al., 1971; Singh et al., 1996). Our study to establish the effect of PHT on the mediators of the first and second phases showed it to possess anti-histaminergic, anti-serotonin and anti-bradykinin properties (Table 5).

Castor oil has been reported to bring about changes in electrolytes and water transport as well as increase in peristaltic activity (Capasso et al., 1986). These changes are associated with prostaglandins that contribute to the pathophysiological functions in the gastrointestinal tract (Bennet and Sanger, 1982). Release of prostaglandins is also a major cause of arachidonic-induced diarrhoea (Luderer et al., 1980), which is characterized by an increased in intestinal transit time and an increase in wet faeces, and the delay of castor oil-induced diarrhoea has been demonstrated to characterize ASA-like drugs (Awounters et al., 1978). However, PHT was found to protect the animals from diarrhoea better than ASA (Table 6). Such an inhibitory effect on the gut motility (unpublished work), further corroborates its likely central effects, since μ_2 – opioid receptor has been reported to mediate inhibition of gut motility (Laurence et al., 1997). A number of biogenic compounds have been identified in PHT. Among these are alkaloids, saponins, tannins, flavonoids and glycosides (Table1). Alkaloids have been found to be responsible for

both analgesic and anti-inflammatory actions in some natural products (Fernanda et al., 2002). Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception (Ramesh et al., 1998; Bittar et al., 2002). Also, there are few reports on the role of tannins in anti-nociceptive and anti-inflammatory activities (Starec et al., 1988). Glycosides, the active component in willow bark have also been found to exhibit inflammatory effects (Ma et al., 1998). Lastly, saponins have been found to inhibit histamine release *in vitro* (Rao and Gurfinkel, 2001). Thus, the presence of these active biological principles in PHT could have accounted for its observed pharmacological actions.

Conclusion

PHT has demonstrated promising anti-nociceptive and anti-inflammatory properties in animal models, confirming its efficacy in the treatment of pain and swellings in its users. However, both central- and peripheral-mediated analgesic mechanisms have been postulated. Further studies, especially at the molecular level may provide better understanding of its exact mode and site(s) of actions.

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